Docket No. 1436-4094
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10, 1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner of Patents
Washington, D.C. 20231

TRANSMITTAL OF DOCUMENTS

Sir:

The following documents are submitted herewith:

1. Supplemental Petition under 37 CFR §1.182;
2. Chang Documentary Exhibit 12;
3. Chang Exhibit 13; and
4 Return receipt postcard

Respectfully Submitted

MORGAN & FINNEGAN

By _____
Eugene Moroz
Reg. No. 25,297

February 28, 1996

Of Counsel:

William S. Feiler
M. Caragh Noone
Dorothy R. Auth
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212) 758-4800
(212) 751-6849 (FAX)

208888_1

Docket No. 1436-4096

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10, 1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner of Patents
Washington, D.C. 20231

SUPPLEMENTAL PETITION UNDER 37 CFR §1.182

Sir:

The attached Chang Documentary Exhibit 12 and Chang Exhibit 13 are filed to supplement the Petition and Amendment sent by Express Mail on February 20, 1996 and hand delivered on February 22, 1996.

Respectfully Submitted

MORGAN & FINNEGAN

February 28, 1996 By Eugene Moroz

Eugene Moroz
Reg. No. 25,237

Of Counsel:

William S. Feiler
M. Caragh Noone
Dorothy R. Auth
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212) 758-4800
(212) 751-6849 (FAX)

208895_1

American Type Culture Collection
12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)881-2600 Telex: 898:055 ATCCNORTH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

National Institutes of Health, National Cancer Institute
Building 37, Room 6A17
9000 Rockville Pike
Rockville, Maryland 20205
Attention: Dr. Flossie Wong-Staal

Deposited on Behalf of: National Institute of Health, National Cancer Institute

Identification Reference by Depositor:	ATCC Designation
λ EH-10 recombinant phage clone of HTLV-III in λ g & Wes λ B	40125
λ EH-5 recombinant phage clone of HTLV-III in λ g & Wes λ B	40126
λ EH-8 recombinant phage clone of HTLV-III in λ g & Wes λ B	40127

The deposits were accompanied by: ___ a scientific description ___ a proposed taxonomic description indicated above.

The deposits were received July 30, 1984 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

___ We will inform you of requests for the strains for 30 years.
X We will not inform you of requests for the strains.
___ The strains are available to the scientific public upon request as of

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same:

The strains will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above were tested March 4, 1987. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC: Bobbie A. Brandon
(Mrs.) Bobbie A. Brandon, Head, ATCC Patent Depository

Date: March 6, 1987
cc: James A. Oliff, Esq.

Form BP 4/9

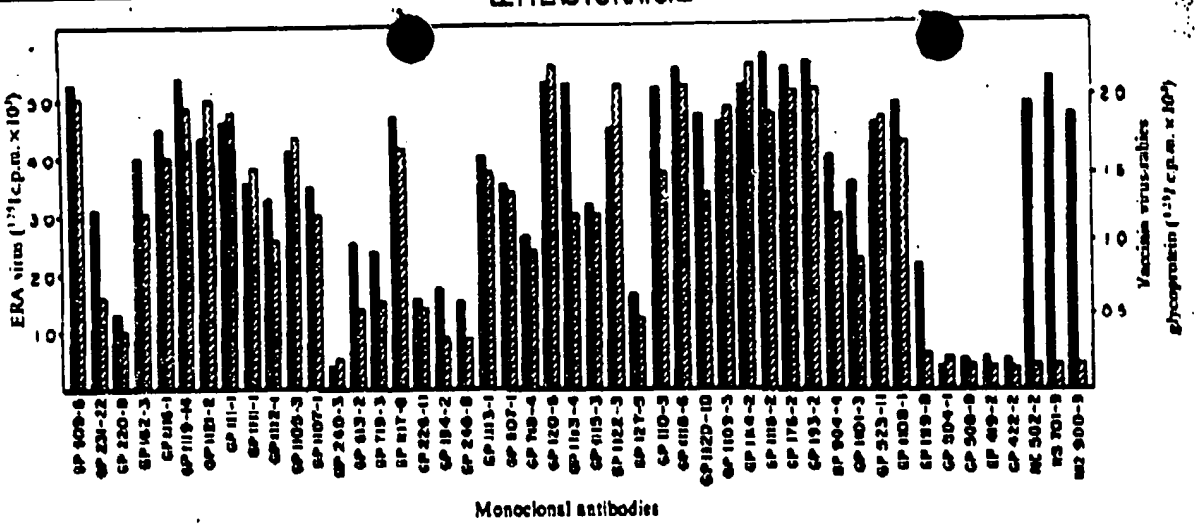


Fig. 3 Comparative binding of VVTGgRAB and ERA virus antigens with a panel of monoclonal antibodies. Solid bars, ERA virus; cross-hatched bars, VVTGgRAB virus.
Methods: Antigens (100 ng) were dried on microtitre plates and treated for 30 min with phosphate-buffered saline (PBS) containing 10% γ-globulin-free horse serum (Gibco). After draining, monoclonal antibody (1:1,000 dilution of ascites fluid; 25 μl) was added, incubated for 1 h at 37 °C and washed with PBS. Each well then received 25 μl of ^{125}I-labelled goat anti-mouse antibodies (30,000 c.p.m., specific activity, 0.5 mCi mg^{-1}). After further incubation (37 °C, 1 h) and washing with PBS, the bottom of each well was cut out and radioactivity determined.

doses of street rabies virus, whereas mice similarly immunized with wild-type VV alone were not protected (Table 2).

To assess the authenticity of the recombinant rabies glycoprotein, reactivity with a panel of monoclonal antibodies directed against rabies glycoprotein and other viral proteins (N, NS and M) was examined. The binding activity of the recombinant glycoprotein with 44 anti-glycoprotein monoclonal antibodies was almost identical to that observed with purified ERA rabies virus, whereas only the ERA virus reacted with anti-N, -NS and -M antibodies (Fig. 3). This demonstrates that the rabies glycoprotein produced by VVTGgRAB virus-infected cells is qualitatively indistinguishable from the native glycoprotein of ERA virus.

Vaccinia virus has been used extensively as a live vaccine to control and eradicate smallpox (see ref. 13 for review); it has been developed as a cloning and expression vehicle for hepatitis B, influenza and herpes antigens and protection has been achieved by vaccination with appropriate influenza- and herpes-VV recombinants[2-4,14]. We demonstrate here that live VV expressing the rabies glycoprotein is capable of conferring protection against experimental rabies infection. Attenuated viruses such as VV are particularly appropriate vehicles for vaccine production: their preparation and administration can avoid costly procedures involving propagation of the pathogenic agent on cultured mammalian cells and subsequent toxicity testing.

We thank A. Kirn and D. Nayak for helpful discussions and P. Chambon, E. Eisenmann and P. Kourilsky for encouragement and critical reading of the manuscript, A. Bailand for preparing the synthetic oligonucleotides used in this work, D. Villeval and F. Jaeger for verifying constructs by sequencing and E. Chambon and F. Daul for assistance in preparing this manuscript. This study was supported in part by NIAID grant AI-09706.

Received 27 July; accepted 27 September 1984.

1. Wiktor, T. J. in *Rhabdoviruses* Vol. 3 (ed. Bishop, D. H. L.) 99–112 (CRC, Boca Raton, Florida, 1980).
2. Smith, G. L., Mackett, M. & Moss, B. *Nature* 302, 490–495 (1983).
3. Smith, G. L., Murphy, B. R. & Moss, B. *Proc. natn. Acad. Sci. U.S.A.* 80, 7155–7159 (1983).
4. Panicali, D., Davis, S. W., Weinberg, R. L. & Paoletti, E. *Proc. natn. Acad. Sci. U.S.A.* 80, 5364–5368 (1983).
5. Anilionis, A., Warner, W. H. & Curtis, P. J. *Nature* 294, 275–278 (1981).
6. Lathe, R., Kieny, M. P., Schmitt, D., Curtis, P. & Lecocq, J. P. *J. molec. appl. Genet.* 2, 331–342 (1984).
7. Yelverton, E., Norton, S., Obijeski, J. F. & Goeddel, D. V. *Science* 219, 614–620 (1983).
8. Panicali, D. & Paoletti, E. *Proc. natn. Acad. Sci. U.S.A.* 79, 4927–4931 (1982).
9. Mackett, M., Smith, J. L. & Moss, B. *Proc. natn. Acad. Sci. U.S.A.* 79, 7415–7419 (1982).
10. Dietzschold, B., Wiktor, T. J., MacFarlan, R. & Varrichio, A. *J. Virol.* 44, 595–602 (1982).
11. Dreika, R. & Spehner, D. *Virology* 131, 383–393 (1983).
12. Dietzschold, B., *J. Virol.* 23, 386–393 (1977).
13. Bernhard, A. M. *Microbiol. Rev.* 47, 455–509 (1983).
14. Paoletti, E., Lipinskas, B. R., Samsonoff, C., Mercer, S. & Panicali, D. *Proc. natn. Acad. Sci. U.S.A.* 81, 193–197 (1984).
15. Smith, J. S., Yager, P. A. & Baer, G. M. *Wld Hlth Org. Monogr.* 33, 354–357 (1973).
16. Lathe, R., Ballard, A., Kohli, V. & Lecocq, J. P. *Gene* 20, 187–195 (1982).
17. Kohli, V. *et al. Nucleic Acids Res.* 10, 7459–7468 (1982).
18. Zoller, M. J. & Smith, M. *Meth. Enzym.* 100, 468–500 (1983).
19. Rodman, M., Wagner, R. E., Glickman, W. & Matsuoka, M. in *Progress in Environmental Mutagenesis* Vol. 7 (ed. Alacevic, M.) 121–130 (Elsevier, Amsterdam, 1980).
20. Kramer, W., Schughart, K. & Fritz, H. J. *Nucleic Acids Res.* 10, 6475–6485 (1982).
21. Lusky, M. & Botchan, M. *Nature* 293, 79–81 (1981).
22. Viera, J. & Messing, J. *Gene* 19, 259–268 (1982).
23. Lathe, R., Kieny, M. P., Skory, S. & Lecocq, J. P. *DNA* 3, 173–181 (1984).
24. Venkatesan, S., Baroudy, B. M. & Moss, B. *Cell* 125, 805–813 (1981).
25. Kieny, M. P., Lathe, R. & Lecocq, J. P. *Gene* 26, 91–99 (1983).
26. Wolf, J. P. & Moss, B. *J. Virol.* 44, 530–537 (1982).
27. Mackett, M., Smith, G. L. & Moss, B. *J. Virol.* 49, 857–864 (1984).
28. Lathe, R., Hirth, P., Dewilde, M., Harford, N. & Lecocq, J. P. *Nature* 284, 473–474 (1980).

Molecular cloning and characterization of the HTLV-III virus associated with AIDS

Beatrice H. Hahn, George M. Shaw, Suresh K. Arya, Mikulas Popovic, Robert C. Gallo & Flossie Wong-Staal

Laboratory of Tumor Cell Biology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

We recently reported the isolation and characterization of a novel human T-lymphotropic retrovirus, HTLV-III, in patients with acquired immune deficiency syndrome (AIDS) and in those at risk for the disease[1–4]. After extensive sero-epidemiological studies[2,5,6], together with numerous virus isolations from these patients[1,7], we concluded that HTLV-III is the causative agent of AIDS. Here we report the molecular cloning and characterization of two highly related but distinct forms of the HTLV-III genome. The viral genome is ~10 kilobases long and is detected in HTLV-III-infected cells but not in uninfected cells, including normal human tissue,

indicating that this virus is exogenous to man. We also demonstrate distant nucleic acid sequence homology between the cloned genome of HTLV-III and those of HTLV-I and HTLV-II. The availability of the cloned HTLV-III genome will now allow an unambiguous comparison of this virus with other retroviruses that also have been associated with the pathogenesis of AIDS[6-11], and moreover, will facilitate the development of diagnostic and therapeutic measures in the treatment of AIDS.

All human retroviruses that have been extensively characterized are lymphotropic, especially OKT4 lymphotropic, and induce formation of multinucleated cells on infection. These viruses also contain a relatively high-molecular weight reverse transcriptase with preference for Mg^{2+} and possess a major core protein of relative molecular mass 23,000-25,000. We named the viruses human T-cell leukaemia viruses, or HTLV, in accordance with recent convention[12,13]. The first two subgroups of HTLV (I and II) are associated with T-cell malignancies and can transform T cells *in vitro*[14]. HTLV-III has many properties in common with HTLV-I and HTLV-II but has cytopathic rather than transforming activity. The crucial step allowing us to isolate and characterize HTLV-III, and to produce sufficient purified viral reagents for serological assays, was the successful transmission of HTLV-III to an immortalized human T-cell line (HT) and to clones derived from this line which were significantly resistant to the cytopathic effects of the virus. This led to the establishment of permanently infected, high-producer cell lines for continuous production of HTLV-III[2]. One of these cell lines, H9/HTLV-III, produces large quantities of HTLV-III and serves as the principal producer cell line for immunological assays used to detect virus-specific antigens and antibodies in sera from AIDS patients. The uninfected parental cell line (HT) and its derivatives (H9 and H4) were negative by all criteria for retrovirus infection, including HTLV-I, HTLV-II and HTLV-III (M.P., in preparation). To clone the HTLV-III genome, we isolated unintegrated viral DNA after acute infection of H9 cells with concentrated HTLV-III and cloned this DNA into a λ phage library to be screened with viral cDNA.

Concentrated virus from the H9/HTLV-III cell line was used to infect fresh uninfected H9 cells at a multiplicity of 50 viral particles per cell and cultures were collected after 4, 10, 15, 24 and 48 h. Extrachromosomal DNA was extracted according to the procedure of Hirt[14] and assayed for its content of unintegrated viral DNA using HTLV-III cDNA as a probe. The synthesis of this cDNA was primed with oligo(dT) and reverse-transcribed from poly(A)-containing RNA of virions that had been banded twice on sucrose density gradients[15]. Unintegrated linear viral DNA was first detected after 10 h and was also present at the subsequent time points. Figure 1 shows a Southern blot of the 15-h sampling. A band of ~10 kilobases (kb) in the undigested DNA represents the linear form of unintegrated

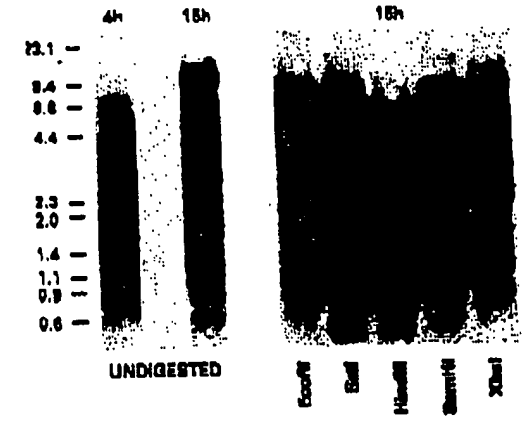


Fig. 1 Southern blot[22] analysis of unintegrated HTLV-III DNA. No viral sequences could be detected in the undigested DNA after 4 h. However, a major species of viral DNA ~10 kb long was present at 10, 15, 24 and 48 h, representing the linear unintegrated form of the virus. The figure shows a representative Southern blot of the 15-h sample digested with several restriction enzymes. Methods: Fresh uninfected H9 cells (8×10^6) were infected with concentrated supernatant from cell line H9/HTLV-III containing 4×10^{11} particles of HTLV-III. Infected cells were divided into five roller boules and collected after 4, 10, 15, 24 and 48 h. Low-molecular weight DNA was prepared using the Hirt fractionation procedure[14] and 30 μg of undigested and digested DNAs were separated on a 0.8% agarose gel, transferred to nitrocellulose paper, and hybridized to an HTLV-III cDNA probe for 24 h at 37 °C in 2.4 × SSC, 40% formamide and 10% dextran sulphate. cDNA was synthesized from poly(A)-selected RNA prepared from doubly banded HTLV-III virus in the presence of oligo(dT) primers[23]. Filters were washed in 1 × SSC at 65 °C.

HTLV-III. No closed or nicked circular DNA could be detected at 10, 15 or 24 h, but both forms were evident in small amounts at 48 h (data not shown). The viral genome was not cleaved by *Xba*I, whereas *Sst*I generated three predominant bands of 9, 5.5 and 3.5 kb (Fig. 1). We interpreted these bands as representing the genomes of two forms of HTLV-III, both cut by *Sst*I in or near the long terminal repeat (LTR), and one having an additional *Sst*I site in the middle of its genome. The other enzymes generated a more complex pattern of restriction fragments requiring cloned DNA for further analysis.

Figure 2 shows the restriction map of three clones, designated λBH10, λBH9 and λBH8, which correspond in size to the three *Sst*I fragments shown in Fig. 1. Comparison of these maps suggests that λBH5 plus λBH8 constitute one HTLV-III

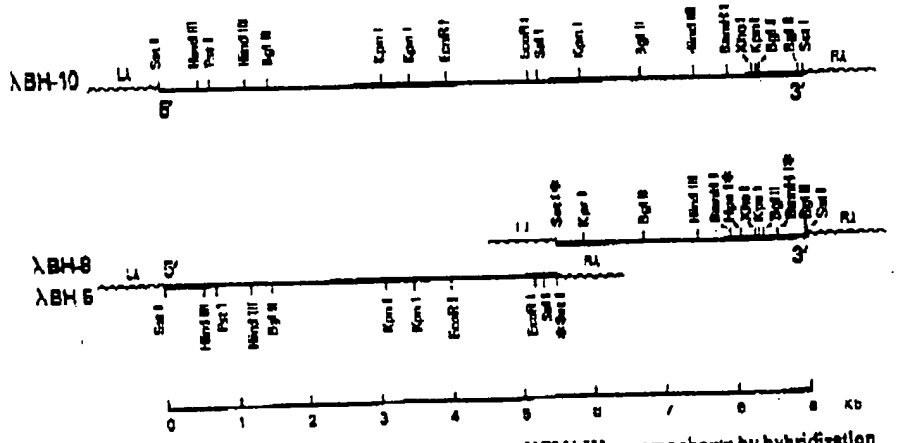


Fig. 2 Restriction endonuclease map of two closely related HTLV-III forms cloned from unintegrated viral DNA. Three recombinant clones (λBH10, λBH5 and λBH8) were analysed and their inserts (9, 5.5 and 3.5 kb, respectively) were mapped with the indicated enzymes. Together they represent two genomic equivalents of HTLV-III that are highly related but differ in three enzyme sites, indicated by bold letters and asterisks.
Methods: Low-molecular weight DNAs pooled from the 15- and 24-h samples were fractionated on a 10–40% sucrose gradient[22]. Aliquots of the fractions were electrophoresed on a 0.5% agarose gel, transferred to nitrocellulose paper and hybridized to HTLV cDNA in conditions described in Fig. 1 legend. Fractions containing the unintegrated linear HTLV-III genome shown by hybridization were pooled; the DNA was subsequently digested with *Sst*I, then ligated to phosphatase-treated *Sst*I arms of λgtWes·λB. After *in vitro* packaging, recombinant phages were screened for viral sequences with HTLV-III cDNA[15,23].

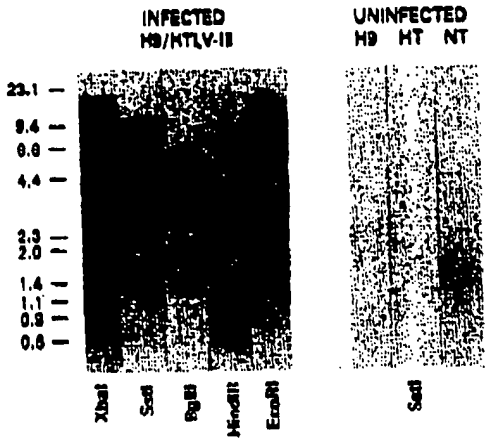


Fig. 3 Demonstration of the presence of HTLV-III viral sequences in the infected cell line, H9/HTLV-III. Both variant forms of HTLV-III defined by differences in *Sst*I sites were detected in H9/HTLV-III DNA. No HTLV-III sequences were found in uninfected H9 cells, uninfected HT cells or normal human thymus (NT). **Methods:** High-molecular weight DNA (10 μg) was digested with restriction enzymes as indicated and hybridized to the nick-translated phage insert from λBH10 in the conditions described in Fig. 1 legend.

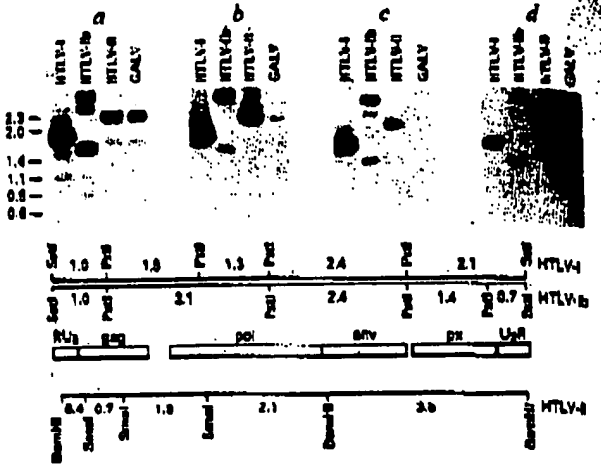


Fig. 4 Sequence homology of HTLV-III to other members of the HTLV family. Schematic restriction maps of HTLV-I, HTLV-Ib and HTLV-II are shown at the bottom, indicating the length (in kb) and location of the generated fragments with respect to the corresponding genomic regions of HTLV-I. LTR, *gag*, *pol*, *env* and pX regions are drawn to scale according to the published nucleotide sequence of HTLV-I[24]. The bands that are most highly conserved as stringency increases correspond to the *gag*/*pol* junction region of HTLV-I (1.8-kb *Pst*I fragment) and HTLV-Ib (3.1-kb *Pst*I fragment) and to the 3′ part of the *pol* region of HTLV-II (2.1-kb *Sma*I/*Bam*HI fragment) which do not overlap assuming that HTLV-II has a genomic organization similar to that of HTLV-I. Fragments corresponding to pX of HTLV-I (2.1-kb *Sst*I/*Pst* fragment) and HTLV-Ib (1.4-kb *Pst* fragment) are only faintly visible at $T_m - 28$ °C on the original autoradiogram. Digestion of GaLV generates six fragments, none of which hybridizes with HTLV-III in medium or high stringency conditions ($T_m = -42$ °C and −28 °C).

Methods: Subclones of full-length genomes of a prototype HTLV-I (unpublished), HTLV-Ib[16], HTLV-II[25] and GaLV (Sesto strain)[26] were digested with the following enzymes; *Pst*I plus *Sst*I (HTLV-I and HTLV-Ib); *Bam*HI plus *Sma*I (HTLV-II); and *Hin*dIII, *Sma*I and *Xho*I (GaLV). Four replicate filters were prepared and hybridized for 36 h under low stringency (8 × SSC, 20% formamide, 10% dextran sulphate at 37 °C) to nick-translated insert of λBH10. Filters were then washed in 1 × SSC at different temperatures: *a*, 22 °C ($T_m - 70$ °C); *b*, 37 °C ($T_m - 56$ °C); *c*, 50 °C ($T_m - 42$ °C); and *d*, 65 °C ($T_m - 28$ °C), and subsequently autoradiographed for 24 h.

genome, and λBH10 another. The two viral forms differ in 3 of 21 mapped enzyme sites, including the internal *Sst*I site. As expected, the phage inserts of λBH5 and λBH8 hybridize in high-stringency conditions ($T_m - 25$ °C) to λBH10 but not to each other, as analysed by Southern blot hybridization and electron microscopic heteroduplex analysis (data not shown). To determine the orientation of the three clones, we used as a probe a cDNA clone (C15) containing U3 and R sequences (S.K.A. *et al.*, in preparation); C15 hybridized strongly to the 0.5 kb *Bgl*II fragment of λBH10 and λBH8, orienting this side 3′. Assuming that *Sst*I cuts only once in the vicinity of the HTLV-III LTR, the clones λBH10 and λBH5/λBH8 represent two complete genomic equivalents of the linear unintegrated form of HTLV-III that vary in three restriction enzyme sites. However, the viral fragments cloned into λBH5 and λBH8 may have been derived from the same or two different viruses.

The presence of two variant forms of HTLV-III in the original cell line was demonstrated by hybridizing the radiolabelled insert of λBH10 to a Southern blot of H9/HTLV-III genomic DNA digested with several restriction enzymes (Fig. 3); both forms were detected using *Sst*I, which generated the expected three bands of 9, 5.5 and 3.5 kb. *Xba*I, which does not cut the provirus, generated a high-molecular weight smear representing polyclonal integration of the provirus, plus a band of ~10 kb. This 10-kb band was also detected in undigested H9/HTLV-III DNA (not shown), indicating that it represents unintegrated viral DNA. The presence of unintegrated viral DNA also explains the 4- and 4.5-kb *Eco*RI fragments seen in both the Hirt and total cellular DNA preparations (Figs 1, 3). Both *Bgl*II and *Hin*dIII cut within the LTR and generate the expected internal bands. Several faint bands in addition to the expected internal bands generated by *Hin*dIII digestion, represent either defective proviruses or other variant forms of HTLV-III present in low copy number.

The absence of HTLV-III sequences from the DNA of the uninfected H9 cell line, the uninfected parental cell line HT and normal human thymus (Fig. 3), demonstrates clearly the exogenous nature of HTLV-III and shows that the virus does not contain human cellular sequences. The same results were obtained using inserts from λBH5 and λBH8 as probes.

The availability of the cloned HTLV-III genome also allowed us to evaluate sequence homology between HTLV-III and other members of the HTLV family including HTLV-I and HTLV-II, as well as a variant of HTLV-I (HTLV-Ib) recently isolated and molecularly cloned from a Zairian patient with adult T-cell leukaemia[16]. Replicate Southern blots of restriction enzyme-digested clones comprising the complete genomes of HTLV-I, HTLV-Ib and HTLV-II, and of gibbon ape leukaemia virus (GaLV) as a control, were hybridized with the full-length HTLV-III probe (BH10) in relaxed conditions, after which the filters were washed in conditions of low, medium and high stringency (Fig. 4). This experiment demonstrates homology between HTLV-III and HTLV-I, HTLV-Ib and HTLV-II, but not between HTLV-III and GaLV. Hybridization of HTLV-III with other members of the HTLV family could be detected in conditions ($T_m - 42$ °C and −28 °C) where no hybridization to GaLV was seen (Fig. 4*c*, *d*). Note that the restriction fragments showing greatest homology to HTLV-III correspond to the *gag*/*pol* region of HTLV-I and to an apparently non-overlapping portion of the *pol* region of HTLV-II (assuming that the genomic arrangement of HTLV-II is similar to that of HTLV-I). Hybridization to a fragment containing exclusively pX sequences in HTLV-Ib (1.4-kb *Pst*I fragment) and to the corresponding fragment in HTLV-I containing pX and LTR sequences (2.1-kb *Pst*I/*Sst*I) was detectable at $T_m - 28$ °C but was very faint. pX sequences of HTLV-II did not hybridize to the HTLV-III probe in the same stringency conditions, nor did fragments containing LTR or envelope sequences of both HTLV-I and HTLV-II.

Overall, these findings using the cloned HTLV-III probe agree with our previous observations using HTLV-III cDNA[16], which also revealed sequence homology, especially in the *gag*/*pol* regions of the HTLV-I, HTLV-II and HTLV-III genomes. However, we emphasise that HTLV-III is much less closely related to HTLV-I and HTLV-II at the nucleic acid level than HTLV-I and HTLV-II are to each other[17,18] and that this homology is most evident in the *gag*/*pol* region of these viruses under stringent hybridization.

Thus, we have molecularly cloned two closely related but distinguishable genomic equivalents of HTLV-III from the H9/HTLV-III cell line, which has been the principal source for all viral reagents used in studies of the sero-epidemiology of HTLV-III in AIDS patients[1-7]. Note that this virus from the H9/HTLV-III cell line retains its cytopathic activity against fresh normal human lymphocytes (unpublished data). Using these clones as probes, we also detected HTLV-III viral sequences in infected cell lines other than H9/HTLV-III that were established from different AIDS patients, and in fresh uncultured lymphoid tissues of AIDS patients[19]. These findings suggest that the cloned HTLV-III genomes reported here represent the probable aetiological viral agent of AIDS. The finding of two variant forms of HTLV-III in the H9/HTLV-III cell line could reflect cumulative *in vitro* mutations in a highly replicative virus. The two forms could also represent different isolates as, when first established, the H9/HTLV-III cell line was infected with pooled material from several different AIDS patients[2]. Preliminary studies of other HTLV-III isolates indeed indicate that HTLV-III, unlike HTLV-I and HTLV-II, exhibits substantial diversity in its restriction enzyme cleavage pattern when isolates from different patients are compared[19]. Further characterization and sequence analysis will help to define the natural variability of this virus, which has important implications with respect to its pathogenicity and origin, and attempts at preventive measures for AIDS. The availability of the cloned HTLV-III genome should also now allow direct comparison of this virus with a similar group of retroviruses described by other investigators[8-11] which has also been linked to the pathogenesis of AIDS and which appears to be immunologically and morphologically indistinguishable from HTLV-III (M. Samgadharan *et al.*, unpublished). Finally, the demonstration of a substantial amount of unintegrated viral DNA in the chronically infected cell line H9/HTLV-III, distinguishes HTLV-III from HTLV-I, HTLV-II and most other retroviruses. It will be important to determine whether the presence of unintegrated DNA has a role in the cytopathicity of HTLV-III, as has been proposed for certain other retroviruses[20,21].

Received 9 August; accepted 27 September 1984.

1. Gallo, R. C. *et al. Science* 224, 500–503 (1984).
2. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. *Science* 224, 497–508 (1984).
3. Sarngadharan, M. G., Popovic, M., Bruch, L., Schupbach, J. & Gallo, R. C. *Science* 224, 506–508 (1984).
4. Schupbach, J. *et al. Science* 224, 503–505 (1984).
5. Safai, B. *et al. Lancet* I, 1438–1440 (1984).
6. Oroszlan, S. E. *et al. N. Engl. J. Med.* (in the press).
7. Salahuddin, S. Z. *et al. Proc. natn. Acad. Sci. U.S.A.* (submitted).
8. Barré-Sinoussi, F. *et al. Science* 220, 868–871 (1983).
9. Montagnier, L. *et al.* in *Human T-Cell Leukemia/ Lymphoma Virus* (eds Gallo, R. C., Essex, M. & Gross, L.) 363–379 (Cold Spring Harbor Laboratory, New York, 1984).
10. Feorino, P. M. *et al. Science* 225, 69–72 (1984).
11. Levy, J. A. *et al. Science* 225, 840–842 (1984).
12. Gallo, R. C., Essex, M. & Gross, L. (eds) *Human T-Cell Leukemia/ Lymphoma Virus* (Cold Spring Harbor Laboratory, New York, 1984).
13. Watanabe, T., Seiki, M. & Yoshida, M. *Science* 222, 1178 (1983).
14. Hirt, B. *J. molec. Biol.* 26, 365–369 (1967).
15. Aye, S. K. *et al. Science* 228, 927–930 (1984).
16. Hahn, B. *et al. Int. J. Cancer* (in the press).
17. Shaw, G. M. *et al. Proc. natn. Acad. Sci. USA* 81, 4646–4648 (1984).
18. Seiki, M. *et al. Science* 225, 431–433 (1984).
19. Shaw, G. M. *et al. Science* (in the press).
20. Kashka, S. & Temin, H. M. *J. Virol.* 31, 376–388 (1979).
21. Weller, S. K., Joy, A. E. & Temin, H. M. *J. Virol.* 33, 494–506 (1980).
22. Southern, E. M. *J. molec. Biol.* 98, 503–517 (1975).
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning: A Laboratory Manual* 32–233 (Cold Spring Harbor Laboratory, New York, 1982).
24. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. *Proc. natn. Acad. Sci. U.S.A.* 80, 3618–3622 (1983).
25. Colombini, S. P., Franchini, G., Mazzetti, V., Wong-Staal, F. & Gallo, R. C. *Proc. natn. Acad. Sci. U.S.A.* 81, 993–997 (1984).
26. Colombini, S. P., Trainor, C. D., Wong-Staal, F. & Reitz, M. S. *J. Virol.* 44, 268–275 (1982).

Metabolic oxidation phenotypes as markers for susceptibility to lung cancer

Rihad Ayesh*, Jeffrey R. Idle†, James C. Ritchie†, Michael J. Crothers† & Martin R. Hetzel*

* The Chest Unit, Whittington Hospital, London N19 5NF, UK
† Department of Pharmacology, St Mary's Hospital Medical School, Paddington, London W2 1PG, UK

That bronchial carcinoma is not an inevitable consequence of cigarette smoking has stimulated the search for host factors that might influence the susceptibility of the individual smoker. One plausible host factor would be a polymorphic gene controlling the metabolic oxidative activation of chemical carcinogens, giving rise to wide inter-subject variation in the generation of cancer-inducing and/or promoting species. Recently, three genetic polymorphisms of human metabolic oxidation have been demonstrated (as characterized by debrisoquine, mephenytoin and carbocysteine), with the metabolism of several substrates exhibiting the phenomenon[1-3]. Debrisoquine 4-hydroxylation segregates into two human phenotypes, each comprising characteristic metabolic capability[4-6]. We report here the frequency of debrisoquine 4-hydroxylation phenotypes in age-, sex- and smoking history-matched bronchial carcinoma and control patients. Cancer patients showed a preponderance of probable homozygous dominant extensive metabolizers (78.8%) with few recessive poor metabolizers (1.6%) compared with smoking controls (27.8% and 9.0% respectively). We conclude that the gene controlling debrisoquine 4-hydroxylation may be a host genetic determinant of susceptibility to lung cancer in smokers and that it represents a marker to assist in assessing individual risk.

The metabolism of debrisoquine was examined in 470 cigarette smokers who had or had not presented with bronchogenic carcinoma, in order to determine the frequency of extensive metabolizer (EM) and poor metabolizer (PM) phenotypes in each group. Patients were recruited from areas of London within the Islington District, Bloomsbury District and Wandsworth District Health Authorities and were admitted primarily to Chest Unit beds at Whittington Hospital. All were white Europeans with a positive history of cigarette smoking (>20 pack-yr, that is, number of packs of 20 cigarettes per day × number of years of smoking). Subjects were excluded if chemotherapy or drugs known to interfere with the phenotyping test[1] had been given, if there were signs of abnormal hepatic or renal function and if additional acute conditions such as heart failure or severe chest infection obtained. The cancer patients ($n = 245$) had a definite diagnosis of bronchogenic carcinoma proven by histology (108), cytology (85) or histology/cytology (44) from samples obtained at bronchoscopy (194), transcutaneous needle biopsy (24), mediastinoscopy (9) and pleural biopsy (6). Cell types comprised squamous cell (138), small cell (68), large cell (8) and undifferentiated (1) carcinomas, together with 30 adenocarcinoma patients. Control patients ($n = 234$) were smokers with chronic airflow limitation, without evidence of carcinoma. Each patient received no drugs after 21.30 h the day before the test, nor for 2 h after the start of the test at 07.00 h. They were each given a 10 mg debrisoquine tablet orally; all urine was collected for the subsequent 8 h and analysed for its content of debrisoquine (D) and 4-hydroxydebrisoquine (4-HD) by electron-capture gas chromatography[7]. The metabolic ratio (urinary D/4-HD) thus determined was used to assign phenotype (EM, 0.1–12.6; PM, 12.7–100)[5]. Routine clinical chemistry and haematology were performed on a blood sample from each patient within 2 days before or after the test.

Cancer and control patients were similar in age (66.3 ± 7.4 (±s.d.) and 67.2 ± 3.3 yr respectively), sex ratio (M/F) (1.82, 1.89) and smoking history (60.3 ± 24.0, 59.4 ± 21.1 pack-yr). The results showed that the patients also had similar levels of plasma Na^+ (137 ± 6, 137 ± 4 mM in cancer and control patients, respectively), HCO_3^- (27.0 ± 5.4, 26.8 ± 4.9 mM), urea (5.0 ± 1.3, 5.1 ±

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10,1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner for Patents
Washington, D.C. 20231

RENEWED PETITION UNDER 37 C.F.R. §1.182

Sir:

Attached is a REQUEST FOR RECONSIDERATION OF THE MARCH 29, 1996 DECISION DISMISSING APPLICANTS' PETITION PURSUANT TO 37 C.F.R. §1.182 TO ADD A REFERENCE TO A PRE-FILING DATE DEPOSIT.

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required in this application, including a petition fee, to Deposit Account No. 13-4500, Order No. 1436-4094. A DUPLICATE COPY OF THIS DOCUMENT IS ATTACHED.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: William S. Feiler
William S. Feiler
Reg. No. 26,728

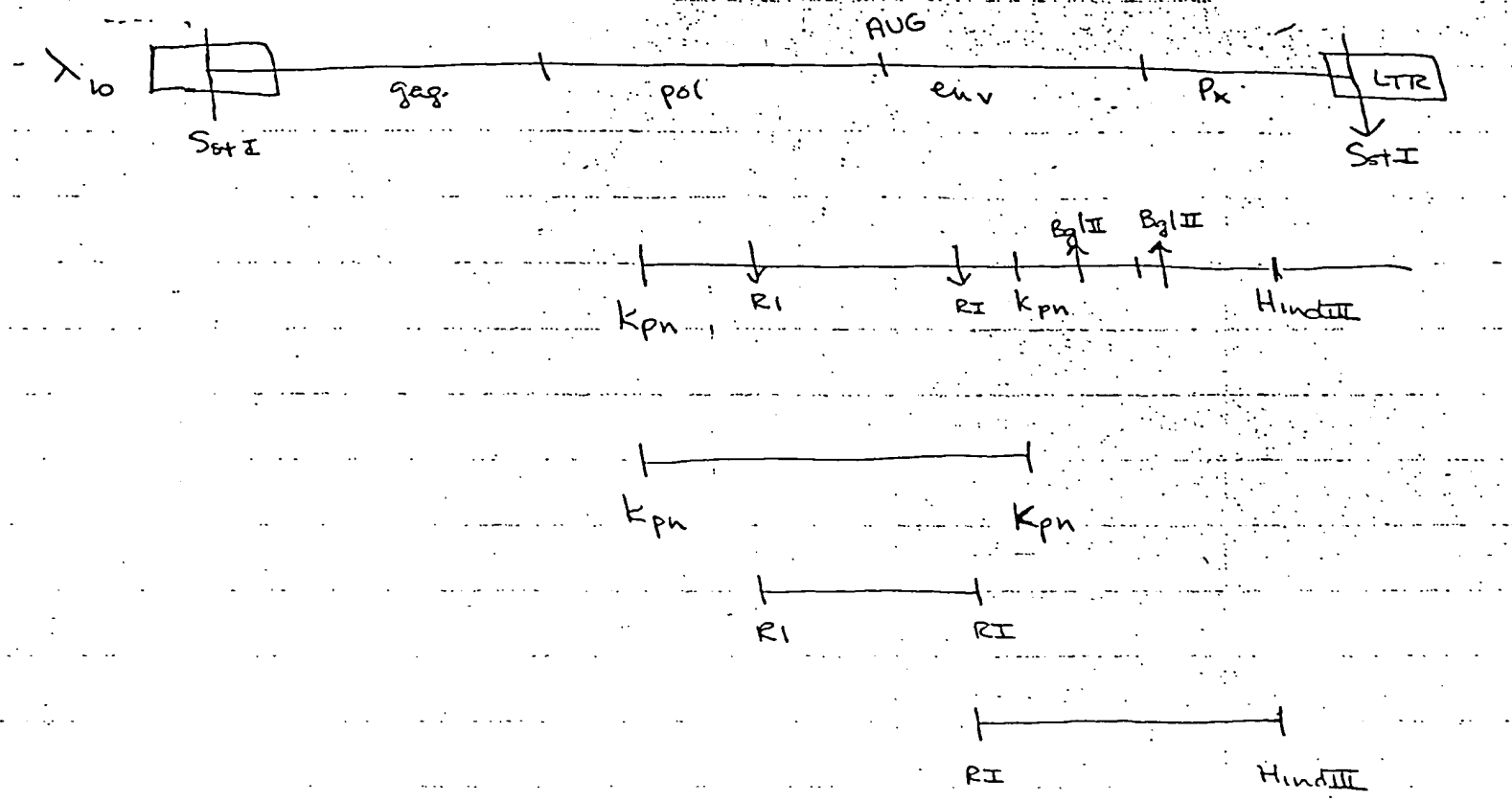
Of Counsel:

Eugene Moroz
M. Caragh Noone
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212)758-4800
(212)751-6849 (FAX)

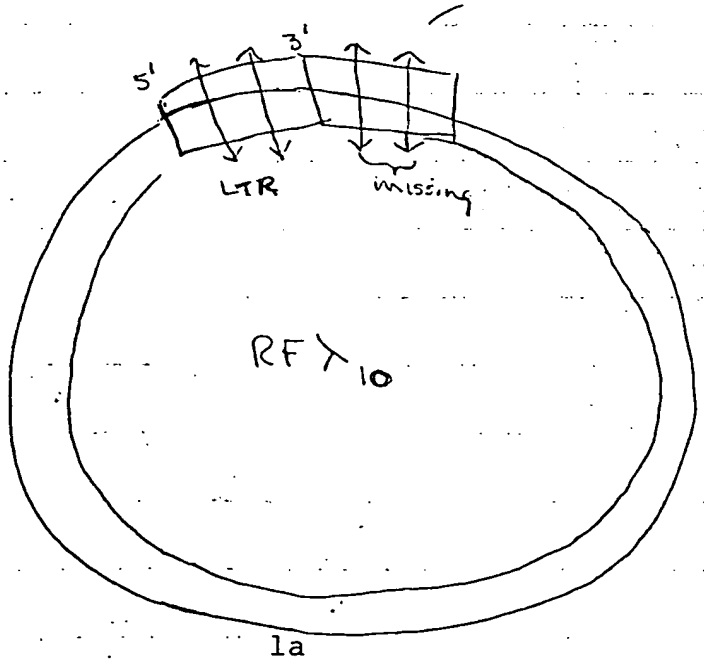
James C. Haight
NATIONAL INSTITUTES OF HEALTH
Office of Technology Transfer
Suite 325
6011 Executive Blvd.
Rockville, MD 20852
(301) 496-7056

659339

FIGURE 1



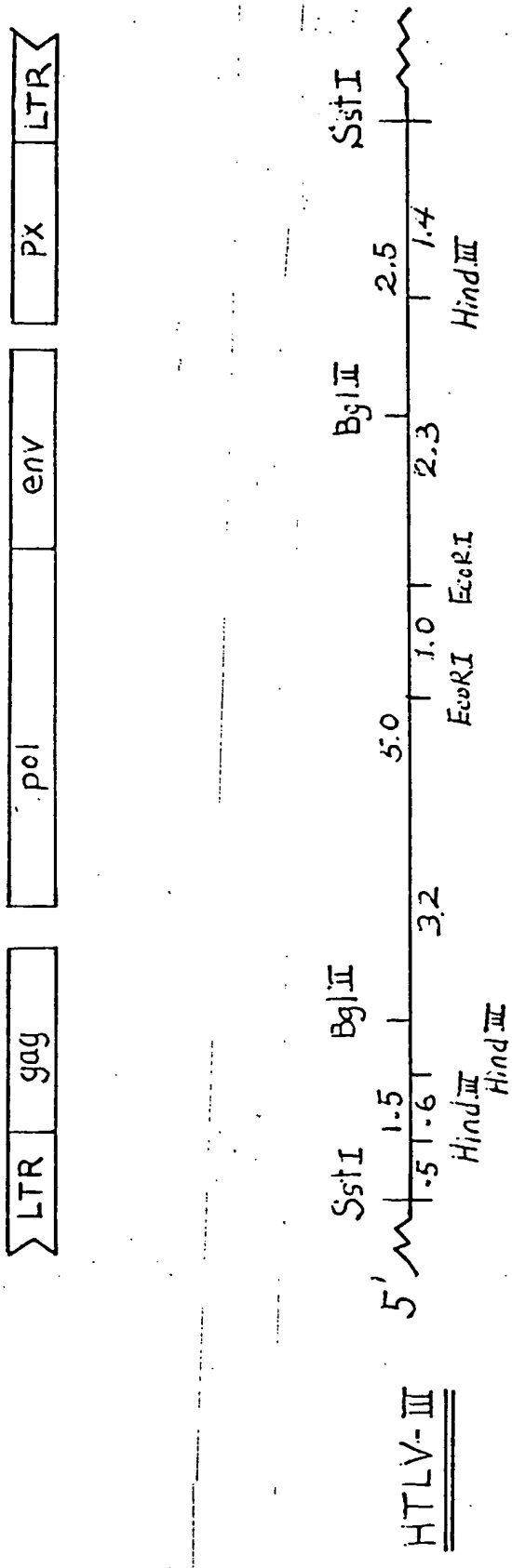
1b



1a

53

FIGURE 2


LTR
gag
pol
env
Px
LTR
5'
3'
SstI
BglII
SstI
BglII
1.5
1.6
.5
3.2
5.0
1.0
2.3
2.5
1.4
HindIII
HindIII
EcoRI
EcoRI
HindIII
HTLV-III

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of : Nancy Chang et al.

Serial No. : 06/659,339

Filed : October 10,1984

For : CLONING AND EXPRESSION OF HTLV-III DNA

Assistant Commissioner for Patents
Washington, D.C. 20231

PETITION UNDER 37 C.F.R. §1.182

Sir:

Applicants are petitioning under 37 C.F.R. §1.182 to amend U.S.S.N. 06/659,339, now abandoned, to include a claim for benefit of co-pending application U.S.S.N. 06/643,306, filed August 22, 1984, and to add a reference to the pre-filing date deposit of a HTLV-III recombinant phage clone referred to in the specification of U.S.S.N. 06/659,339. A proposed amendment is submitted herewith together with a check in the amount of $130.00 to cover the petition fee.

STATEMENT OF FACTS

1. U.S.S.N. 06/659,339 (the "'339 application") was filed on October 10, 1984. Drs. Nancy Chang, Flossie Wong-Staal

and Robert Gallo are the inventors[1]. It was abandoned in favor of U.S.S.N. 06/693,866 ("the '866 application"), a continuation-in-part application filed on January 23, 1985. The '866 application is pending and is currently involved in Interference No. 102,822 (APJ Andrew Metz).

2. The '339 application is the grand parent application for U.S.S.N. 08/080,387 (the '387 application") filed on June 21, 1993. The '387 application is currently involved in Interference No. 103,659 (APJ Michael Sofocleus). Applicants are the Senior Party. Chiron Corporation is the real party in interest for the Junior Party.

3. U.S.S.N. 06/643,306, directed to Molecular Clones of the Genome of HTLV-III, was filed on August 22, 1984. This application describes the cloning of HTLV-III from an immortalized human T-cell line and the preparation of molecular clone λBH-10. Drs. Flossie Wong-Staal, Robert C. Gallo, Beatrice Hahn and Mikulas Popovic are the inventors. The '339 application was co-pending

---

[1] As filed, the '339 application listed Dr. Nancy Chang as the sole inventor. On May 14, 1986, petitions to change the inventorship to add Dr. Robert Gallo and Dr. Flossie Wong-Staal were filed in the '339 application and in U.S.S.N. 06/693,866, the continuation in part application filed on January 23, 1985. Apparently, the '339 application was abandoned before the petition to change inventorship was acted upon. However, in Paper No. 13, issued November 27, 1987, the PTO examiner changed the inventorship of the '866 application to include Dr. Gallo and Wong-Staal. Pursuant to the Weil v. Fritz, 572 F.2d 856 (C.C.P.A. 1978) and In re Schmidt, 293 F.2d 274 (C.C.P.A. 1961) decisions, amendment of the '866 application was legally effective to change the inventorship of the '339 application. Thus, Drs. Chang, Gallo and Wong-Staal are the legal inventors of the '339 application.

with U.S.S.N. 06/643,306 and shares two common inventors, namely, Drs. Gallo and Wong-Staal.

4. Prior to the filing date of the '339 application, recombinant phage clones harboring HTLV-III DNA designated λBH-5, λBH-8 and λBH-10 were deposited by Dr. Flossie Wong-Staal an inventor of the '339 application. On July 30, 1984 these clones were received by the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, 20852, and accepted for deposit under ATCC accession numbers 40126, 40127 and 40125, respectively. The ATCC form recognizing the deposit and its acceptance is attached as Chang Documentary Exhibit 12. The deposit is in full compliance with PTO rules.

5. Clone λBH-10 was specifically identified in the '339 application as set forth in detail in the attached proposed amendment.

6. On September 14, 1995, the United States District Court for the Northern District of California issued a decision in the action captioned Chiron Corporation v. Abbott Laboratories, Civil Action C-93-4380(MHP). The Applicants were not parties to the action. Abbott Laboratories is a licensee of the Applicants under the Chang applications. The decision is reported at 902 F. Supp. 1103 (N.D.Cal. 1995) (the "California Decision") and is attached as Chang Documentary Exhibit 1.

7. In the California Decision, the court, without the benefit of any expert testimony and on a record which the court characterized as "quite weak", found that the '339 application does

not enable one of ordinary skill to obtain or make the starting material, i.e., the HTLV-III clones (the "starting material finding"). Chang Documentary Exhibit 1, 902 F. Supp at 1126.

8. In the California Decision, the Court noted that Chiron had asserted that the '339 application did not indicate that the inventors possessed a means for making a recombinant clone encoding the env region of HTLV-III. The court made no finding on this issue (the "written description issue"). Chang Documentary Exhibit 1, 902 F. Supp at 1128-1129.

9. In the California Decision, the court again, on a very limited record, found that the '339 application fails to set forth the best mode based upon the absence of an enabling disclosure regarding the starting material, i.e., HTLV-III clones (the "best mode finding"). Chang Documentary Exhibit 1, 902 F. Supp. at 1129.

REASONS FOR GRANTING PETITION

The petition to enter these amendments in the '339 application should be granted because the amendments are in accordance with PTO rules and practice and Federal Circuit precedent and may facilitate resolution of issues in the interferences.

The amendment seeking to add the specific reference to the '306 application is appropriate under 35 U.S.C. § 120. The '306 application was filed by two inventors common to this application and was co-pending. The '306 application describes the

cloning of HTLV-III and the preparation of a molecular clone of HTLV-III used in the '339 application. The amendment seeks to add a specific reference to the earlier filed '306 application. Since the '339 application is abandoned, a petition to the Commissioner is appropriate. Under the authority of Sampson v. Commissioner of Patents, 195 U.S.P.Q. 136 (D.C.D.C., 1976), entry of the amendment to the '339 application is appropriate.

The amendment to the application adding the reference to the deposit of the HTLV-III clone at the ATCC is also proper under In Re Lundak, 773 F.2d 1216 (Fed. Cir. 1985). As the Court noted:

> Constructive reduction to practice does not turn on the question of who has possession of a sample, and thus it does not turn on the inclusion or absence, in the specification as filed of the name and address of who will have possession of the sample on grant of the patent.
>
> * * *
>
> We conclude that .... the insertion of depository data after filing is not new matter under 35 U.S.C. § 132.

773 F.2d at 1223. The Court of Appeals further noted:

> [T]he function of section 112 in ensuring *complete public disclosure* is only violated if the *disclosure* is not complete *at the time it is made public* i.e. at the issue date.

773 F.2d at 1223 (citations omitted).

The entry of these amendments is warranted in equity to address the starting material finding, the written description issue and the best mode finding in the California Decision, which

Chiron will undoubtedly raise in the interference. The California findings are erroneous, particularly in light of the deposit and resulting availability of the starting material, which is specifically identified in the '339 application, and the description in the '306 application of the molecular cloning of the HTLV-III starting material. The entry of the amendments are fully warranted under controlling law. Accordingly, entry of the proposed amendment is fully justified.

CONCLUSION

Applicants respectfully request that the petition be granted and that the amendment to the '339 application be entered to protect Applicants' patent rights.

AUTHORIZATION

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required in this application,

including a petition fee, to Deposit Account No. 13-4500, Order No. 1436-4094.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

By: Eugene Moroz
Eugene Moroz
Reg. No. 25,237

Of Counsel:

William S. Feiler
M. Caragh Noone
Dorothy R. Auth
MORGAN & FINNEGAN, L.L.P.
345 Park Avenue
New York, New York 10154
(212)758-4800
(212)751-6849 (FAX)

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail in an envelope addressed to: the Assistant Commissioner for Patents, Washington, D.C., 20231, on February 20, 1996.

Dated: February 20, 1996 By: Eugene Moroz

Eugene Moroz

198026_1

American Type Culture Collection
12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)881-2600 Telex: 898-055 ATCCNORTH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

National Institutes of Health, National Cancer Institute
Building 37, Room 6A17
9000 Rockville Pike
Rockville, Maryland 20205
Attention: Dr. Flossie Wong-Staal

Deposited on Behalf of: National Institute of Health, National Cancer Institute

Identification Reference by Depositor:	ATCC Designation
λ EH-10 recombinant phage clone of HTLV-III in λg & Wes λB	40125
λ EH-5 recombinant phage clone of HTLV-III in λg & Wes λB	40126
λ EH-8 recombinant phage clone of HTLV-III in λg & Wes λB	40127

The deposits were accompanied by: ___ a scientific description ___ a proposed taxonomic description indicated above.

The deposits were received July 30, 1984 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

___ We will inform you of requests for the strains for 30 years.
_X_ We will not inform you of requests for the strains.
___ The strains are available to the scientific public upon request as of

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same:

The strains will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above were tested March 4, 1987. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC: Bobbie A. Brandon
(Mrs.) Bobbie A. Brandon, Head, ATCC Patent Depository

Date: March 6, 1987
cc: James A. Oliff, Esq.

Form BP 4/9

P.3

NOV 22 '95 10:33AM ATCC

Documentary Exhibit 12
CHANG ET AL.
Interference No. 103,659

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.